Estrogen Regulation of Epididymal Sperm Maturation

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Background

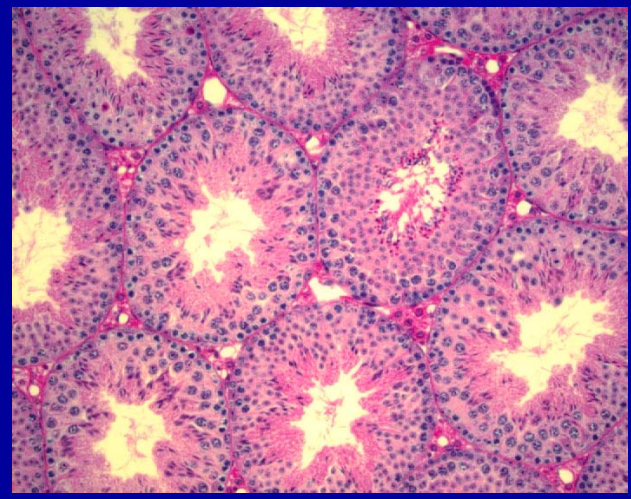
- The target tissues of estrogens and the specific functions of estrogen receptors in male reproductive function are unknown.
- Estrogen receptor α (ERα) and β (ERβ) are expressed in a wide variety of cell-types in the male reproductive tract.
- The highest levels of ERα expression are found in the epithelial cells of the efferent ducts and caput region of the epididymis in rodents.
- αERKO mice are infertile, while βERKO mice are fertile.
- Infertility of αERKO males is due to disruption of ER action within somatic cells and not germ cells (Mahato et al., 2000).

αERKO Male Mice Are Infertile

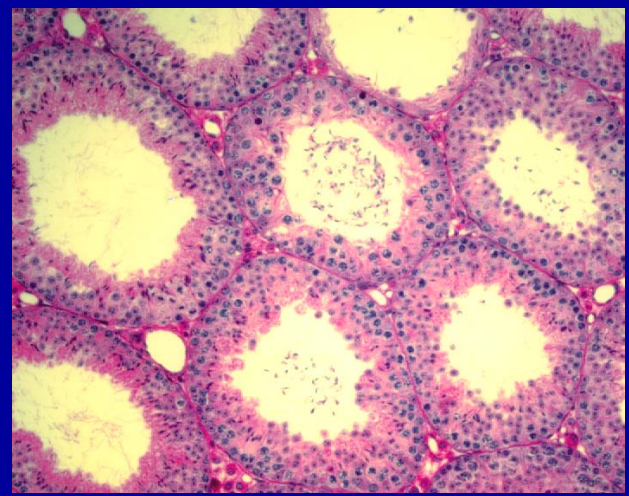
ERα -/- phenotype:

- Altered testicular morphology
- Dilated rete testis
- Reduced mating frequency
- Reduced epididymal sperm count
- Reduced sperm motility
- Sperm fail to fertilize eggs *in vitro*

Wild Type

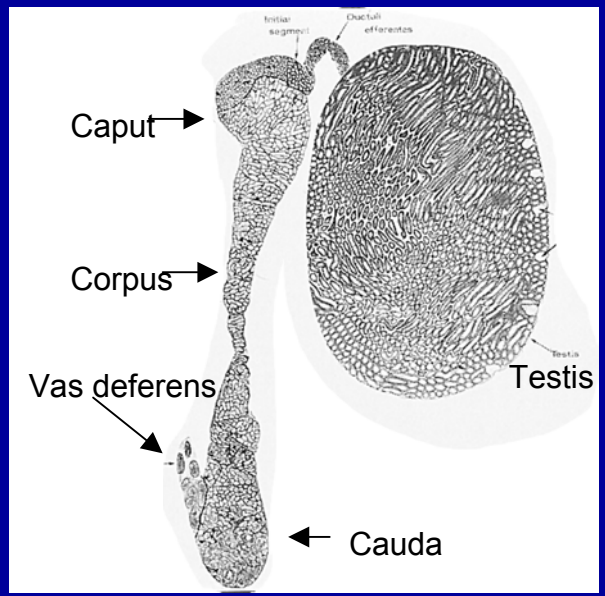


αERKO



Epididymis

- Functions as a storage site and in the final maturation of sperm.
- ERα is present in epithelial cells with highest levels in the caput.
- Epithelial cells modify the luminal fluid and alter biochemical and physiological properties of sperm.
- Sperm maturation and transport are reported to be influenced by estrogens, but few studies have been done.



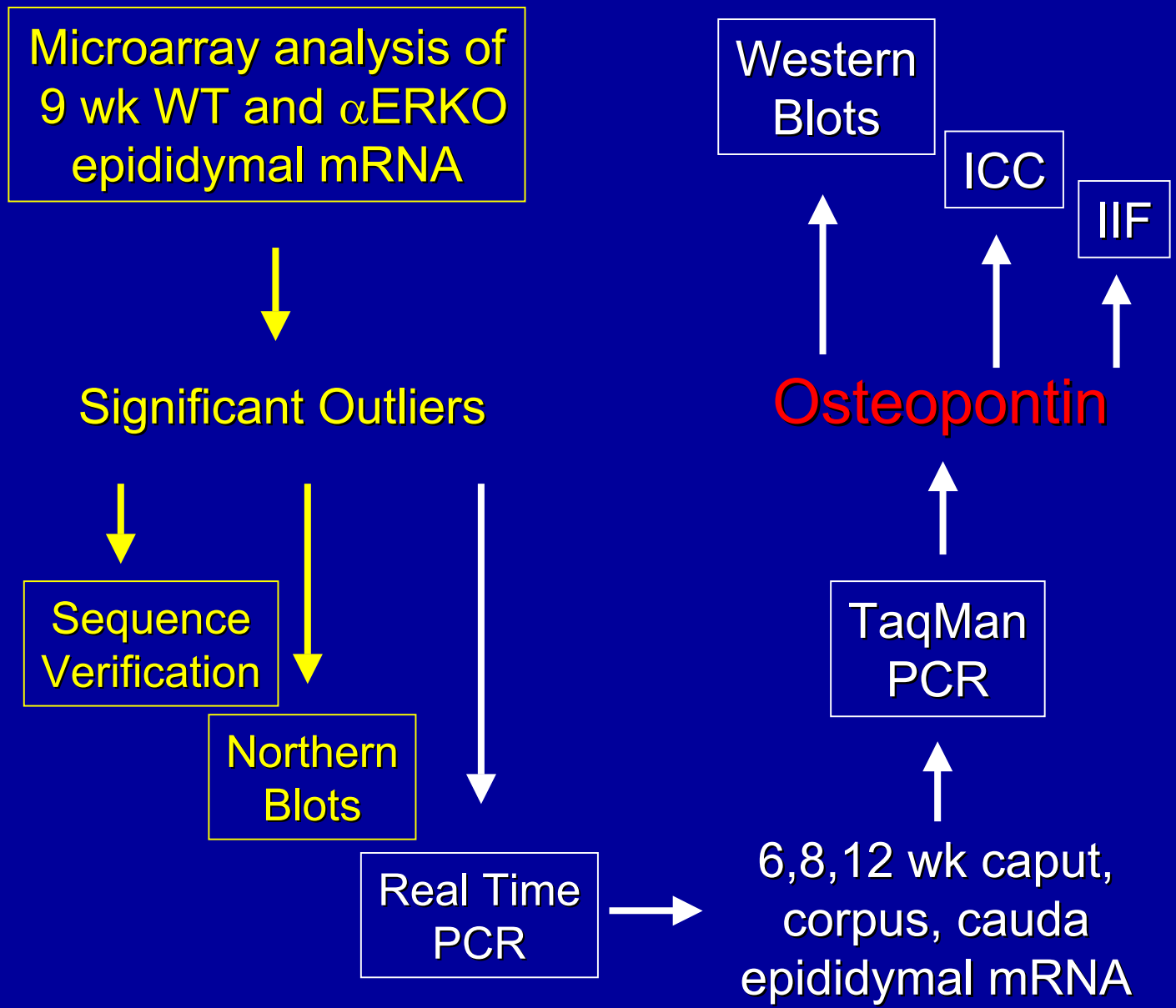
Cauda sperm of αERKOs exhibit low motility and fail to fertilize eggs suggesting that sperm of αERKO mice fail to undergo epididymal maturation.

Rationale: Sperm acquire motility and the ability to fertilize eggs as they transit the epididymis. Epididymal sperm of αERKO mice fail to undergo these changes. Therefore, it is suggested that ERα and estrogens play a fundamental role in male reproduction by regulating critical aspects of epididymal sperm maturation.

Hypothesis: Estrogen regulates the synthesis of proteins by epididymal cells required for sperm to undergo the physiological changes and surface modifications necessary to become capable of fertilization.

Goal: To identify estrogen-regulated proteins that are involved in the epididymal maturation of sperm and determine their roles in this process.

Experimental Design

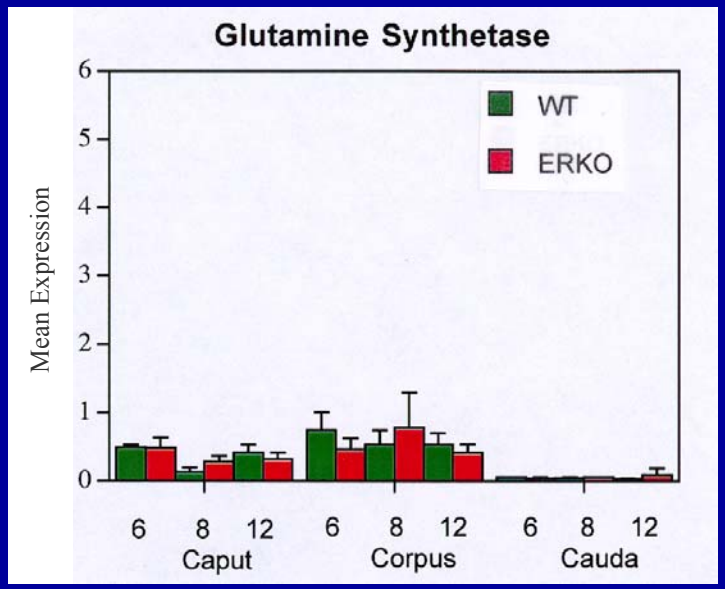


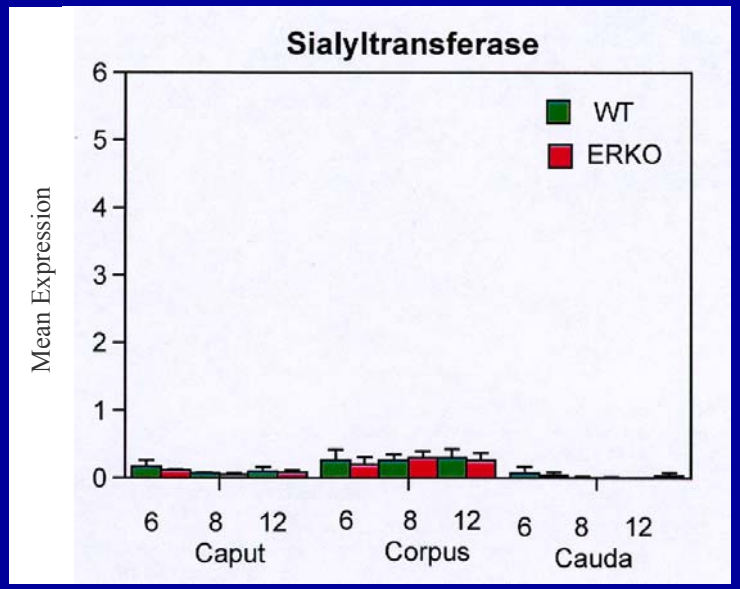
	Expression Levels			
Clone Identification	**Microarray**	**Northern**	**Real Time PCR**	**Sequence**
Hemaglobin alpha	2.1x ERKO	.9x ERKO		NO sequence
EST	1.7x ERKO	2.3x ERKO		YES
EST	1.6x ERKO			NO sequence
EST	1.6x ERKO	1.7x ERKO		NO
Osteopontin	1.5x ERKO	2.1x ERKO	4.3x ERKO	YES
Thymopoietin alpha	1.5x ERKO	1.8x ERKO		YES
EST	1.4x ERKO			NO sequence
Mus Xlr4 mRNA	1.4x ERKO	2.3x ERKO		YES
mRNA for ribonucleoprotein H1	.7x ERKO	1.2x ERKO		YES
Solute carrier family 31	.7x ERKO	.8x ERKO		NO
Heat shock protein cognate 70	.7x ERKO			YES
EST	.7x ERKO			YES
Phosphodiesterase	.7x ERKO	.9x ERKO		YES
Calcium binding protein	.7x ERKO			NO sequence
EST	.6x ERKO	.9x ERKO		NO
Glutamine synthetase	.6x ERKO	.9x ERKO		YES
EST	.6x ERKO	.7x ERKO		YES
EST	.6x ERKO	.7x ERKO		YES
Sialyltransferase	.6x ERKO	.4x ERKO	.6x ERKO	YES
SSeCKS, testis specific gene A1	.6x ERKO	.4x ERKO	.5x ERKO	YES
ATP-binding cassette	.6x ERKO	.8x ERKO		YES
Metallothionein 2	.5x ERKO	.7x ERKO		NO

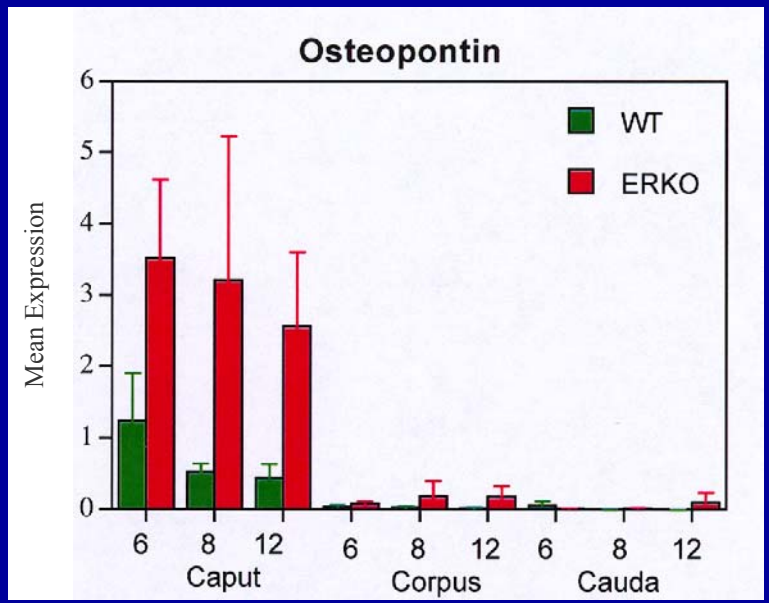
Objective: To measure differential expression of selected genes by collecting caput, corpus, and cauda epididymidies from 6, 8, and 12 week WT and αERKO males for real time PCR analysis.

Hypothesis: The caput, corpus and caudal regions of the epididymis will show differential gene expression and the results gathered from microarray analysis will be enhanced following real time PCR analysis.

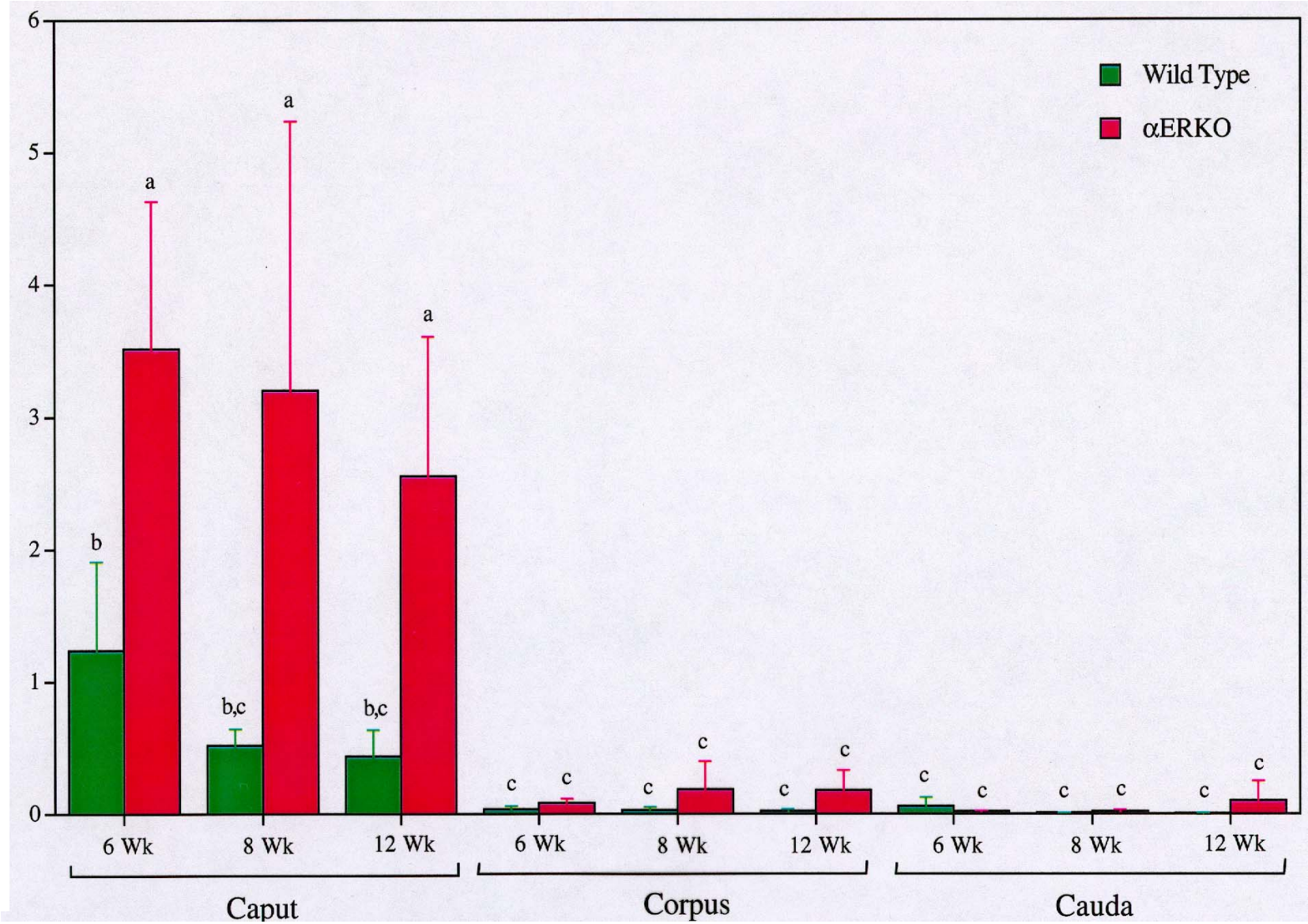
TaqMan PCR


Glutamine Synthetase
Mean Expression
WT
ERKO
0
1
2
3
4
5
6
6 8 12 6 8 12 6 8 12
Caput
Corpus
Cauda


Sialyltransferase
Mean Expression
WT
ERKO
0
1
2
3
4
5
6
6 8 12 6 8 12 6 8 12
Caput
Corpus
Cauda


Osteopontin
Mean Expression
WT
ERKO
0
1
2
3
4
5
6
6 8 12 6 8 12 6 8 12
Caput
Corpus
Cauda

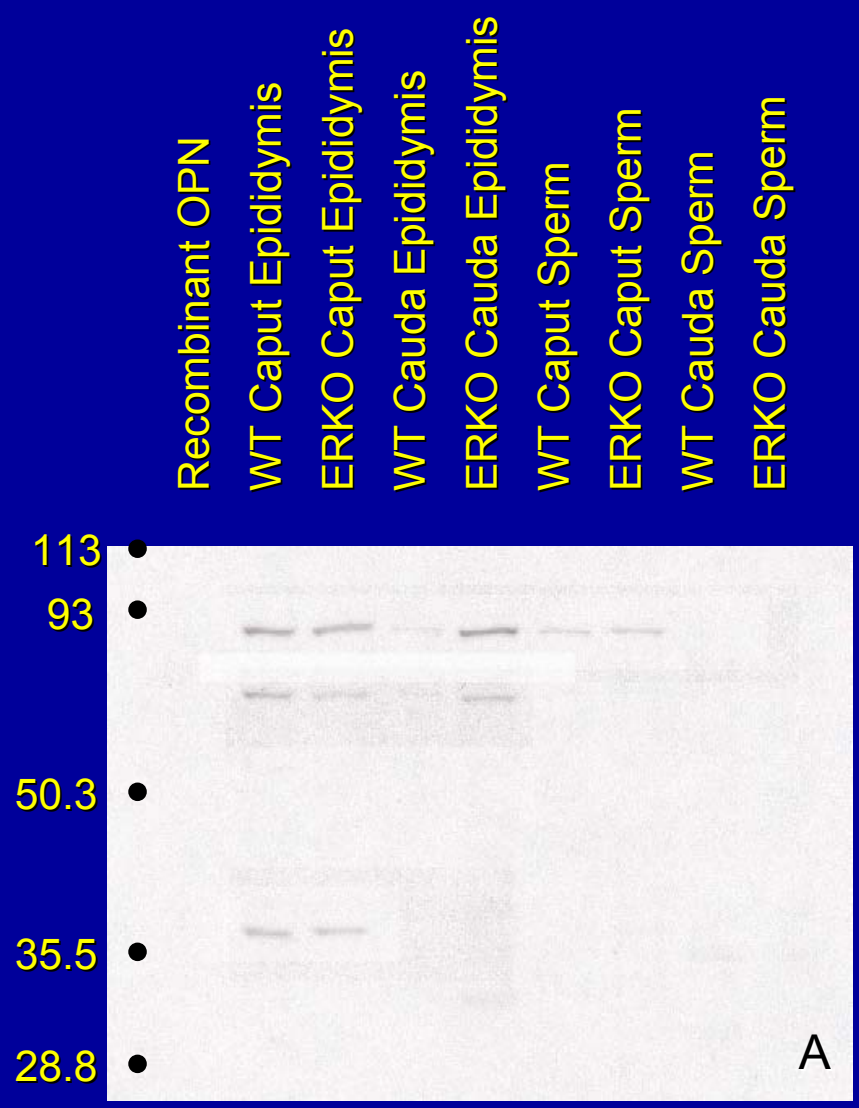
Osteopontin Gene Expression

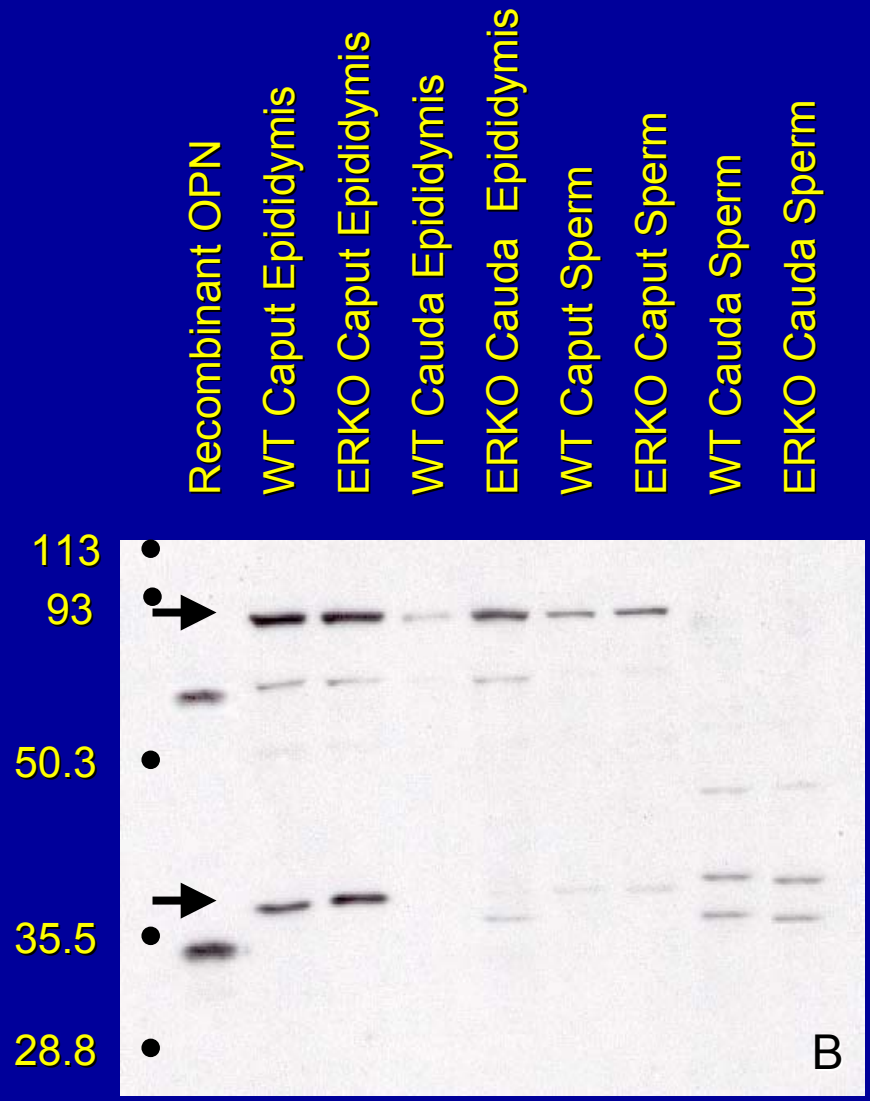


Osteopontin

- Originally isolated from bone matrix and is the principal phosphorylated glycoprotein of bone.
- It is expressed in a wide variety of mammalian tissues and biological fluids.
- Highly acidic, calcium binding, secreted phosphorylated glycoprotein.
- MW has been reported to range from 31-75 KDa due to post-translational modification and splice variants.
- Single copy gene with two half-EREs.

Western Blot





Osteopontin and Male Reproduction

- OPN has been identified in rat epididymis and on rat and bull epididymal sperm.
- It has been reported that the immunoreactivity of OPN on the sperm head in rats disappears as sperm transit the epididymis (Sitteri et al., 1995).
- It has been suggested that the presence of OPN, a calcium-binding protein, in the epididymis may serve as a "decapacitation" factor to prevent premature activation of epididymal sperm motility or fertilizing ability (Sitteri et al., 1995).
- Hypothesis: over-expression of OPN in αERKO males may lead to the reduced sperm motility and the inability to fertilize eggs.

Conclusions

- 22 genes were found to be differentially expressed in 9 wk WT and αERKO epididymides following microarray analysis.
- Based on validated microarray results and potential relevance to epididymal function and estrogen regulation, 3 genes were chosen for further exploration using TaqMan PCR.
- TaqMan PCR indicated that osteopontin was up-regulated in the caput region of the epididymis in αERKOs compared to WT.
- Western blot analysis has indicated OPN protein is expressed in the caput and cauda regions of WT and αERKO animals with an increase in expression in the cauda region of αERKOs compared to WT animals.

Future Studies

1. Complete Immunohistochemistry to localize OPN protein expression in the epididymis.

2. Examine OPN protein expression on epididymal sperm by using indirect immunoflourescence.

3. Microarray analysis of WT and αERKO caput epididymis to determine if additional differentially expressed genes can be determined.

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